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Enhancement of Osmotic- and Hypovolemic-Induced Drinking by Chlordiazepoxide in Rats Is Blocked by Naltrexone

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COOPER, S. J. *Enhancement of osmotic- and hypovolemic-induced drinking by chlordiazepoxide in rats is blocked by naltrexone*. PHARMAC. BIOCHEM. BEHAV. 17(5) 921–925, 1982.—Recent reports indicate that benzodiazepine-induced hyperphagia can be antagonised by naloxone, an opiate antagonist. Benzodiazepines are also known to facilitate water ingestion in water-deprived rats, and the present study showed that in addition, benzodiazepine treatment can enhance drinking which is elicited by an osmotic thirst stimulus (2 M hypertonic saline) or by a hypovolemic thirst stimulus (20% polyethylene glycol). In both cases, low dose levels of naltrexone (also an opiate antagonist) dose-dependently suppressed the facilitation of thirst-aroused drinking by chlordiazepoxide. Taken with recent biochemical data these behavioral results indicate that the enhancement of ingestive responses by benzodiazepines may depend upon a naloxone-reversible release of endogenous opioid peptides.

Chlordiazepoxide Drinking Hypovolemic thirst Osmotic thirst Naltrexone

THE behavioral and central biochemical effects of benzodiazepines may involve the activation of endogenous opioid mechanisms. Naloxone, an opiate receptor antagonist, attenuates the hyperphagia induced by diazepam treatment both in the rat and the hamster [3, 4, 36, 37]. Naloxone has also been shown to cancel the anticonflict effect of diazepam, which can be measured in terms of an enhanced responding for food in the presence of aversive footshock [17,36]. Furthermore, naloxone has been reported to block the enhancement of lateral hypothalamic self-stimulation responding produced by chlordiazepoxide [27]. Lorens and Sainati [27] suggested that chlordiazepoxide may enhance the self-stimulation responding by releasing an endogenous opioid peptide which acts at opiate receptors. Recent biochemical data indeed confirm that acute benzodiazepine treatment brings about a rapid decrease in striatal enkephalin levels, possibly due to a drug-induced release of peptides [18, 19, 39]. The changes in striatal enkephalin levels brought about by benzodiazepine action are reversible by naloxone [19].

Benzodiazepines not only induce hyperphagia [8], but also promote increased drinking in water-deprived animals [10, 11, 12, 14, 28, 29, 30, 34, 35]. There is some evidence that this hyperdipsic action of benzodiazepines may also involve the release of endogenous opioid peptides, since treatment with either naloxone (1 and 10 mg/kg) or naltrexone (0.1–10 mg/kg) completely abolished the increased drinking brought about by chlordiazepoxide [12]. The pharmacological specificity of this effect was demonstrated by the finding that phenobarbital-induced hyperdipsia in water-deprived rats was not attenuated by either naloxone or naltrexone (0.1–10 mg/kg, in each case) [13]. The physiological specificity of the effect may be investigated through consideration of water deprivation as a thirst stimulus. Water deprivation results in the depletion of both the intracellular and the extracellular fluid compartments; depletion of either gives rise to a thirst signal [22,32]. An interesting question to consider is whether or not benzodiazepine treatment enhances the drinking that follows from either source of thirst. If benzodiazepine treatment does increase drinking in response to intracellular and extracellular thirst challenges, then the next question to consider is whether or not the effects can be countered by the action of an opiate receptor antagonist. Both questions were addressed in the present study, and in the first experiment drinking was elicited in response to the cellular dehydration produced by injection of hypertonic saline solution [20,24]. In the second experiment, drinking was elicited in response to extracellular dehydration brought about by injection of polyethylene glycol [21,38]. In both experiments, the aims were, first, to investigate if the drinking could be enhanced by chlordiazepoxide treatment, and second, to investigate if naltrexone could reverse any chlordiazepoxide effect. Naltrexone was selected for the study because there is evidence that it exerts effects on ingestive responses over a relatively long duration [1,23]. Furthermore, many of the previous

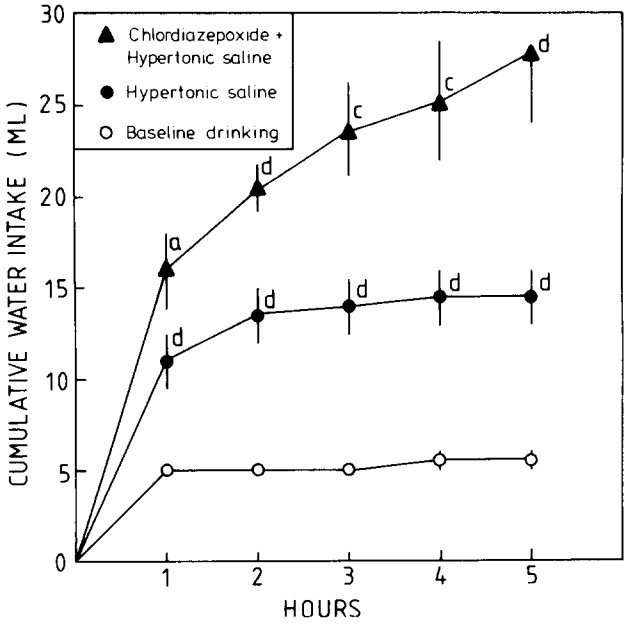

FIG. 1. The cumulative water intake (ml) following injection of 2 M hypertonic saline (●), hypertonic saline and chlordiazepoxide (▲), or in control rats showing baseline drinking (○), at hourly intervals over a 5 hr test. Results are shown as mean±S.E.M. (n=7 per group). Groups treated with naltrexone are not shown. Levels of significance in statistical comparisons (one-tailed t-test): (a) $p<0.05$, (b) $p<0.025$, (c) $p<0.01$, (d) $p<0.005$. In the case of animals treated with hypertonic saline (●), the comparisons are with the baseline control rats. In the case of animals treated with chlordiazepoxide (▲), the comparisons are with the (●) rats.

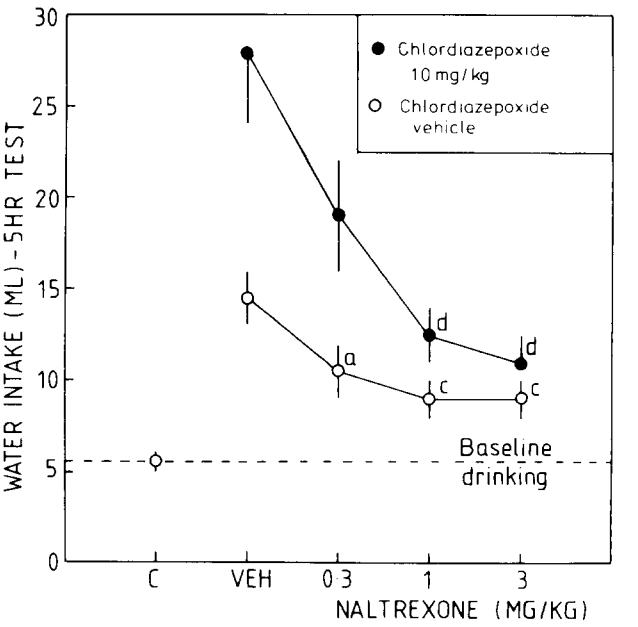

FIG. 2. Antagonism of chlordiazepoxide-enhancement of osmotic thirst elicited by 2 M hypertonic saline after naltrexone (0.3–3 mg/kg). Water intake (ml) over a 5 hr test (mean±S.E.M. shown, n=7 per group). Horizontal interrupted line shows intake of control rats showing baseline drinking. Statistical results (one-tailed t-test) are shown for naltrexone data compared with corresponding vehicle (VEH) levels of water intake. Levels of significance, as for Fig. 1.

studies concerned with benzodiazepine-opiate antagonist interactions have relied on naloxone; using naltrexone would help to establish the generality of such interactions.

EXPERIMENT 1

METHOD

Animals

The subjects were 63 male hooded rats (General strain) bred in this laboratory. They were housed individually in stainless steel cages, with free access to food pellets (Diet 41B, Heygate and Sons, U.K.). They were maintained under a 12 hr light-12 hr dark cycle (lights on at 7 p.m.) and room temperature was kept constant at 21°C. Care was taken to familiarize the animals completely with handling and drug injection procedures before running the drug trials. They weighed 350–450 g at testing.

Procedure

The rats were allocated randomly to 9 groups (n=7 per group). Each rat received 3 injections prior to the 5 hr drinking test. Animals in eight of the groups received an intraperitoneal injection of 2 ml 2 M sodium chloride solution, given 2 hr before the start of the drinking test. In four of these groups, the rats received an intraperitoneal injection of 10 mg/kg chlordiazepoxide HCl, 40 min before the drinking test, whilst animals in the other four groups received a corresponding control injection of isotonic saline. The dose of chlordiazepoxide was chosen as the optimal value which produced hyperdipsia in earlier studies [11, 30, 34]. Each of the four groups in the chlordiazepoxide and in the vehicle conditions were then injected subcutaneously with naltrexone HCl at one of 4 dose levels: 0, 0.3, 1 and 3 mg/kg, respectively. These injections were administered 20 min prior to the beginning of the drinking test. The ninth group of rats received equivalent volumes of isotonic saline corresponding to each of the three injection procedures. These animals were not therefore challenged with the osmotic thirst stimulus, and their data provided the baseline water intake against which to compare the response of the challenged rats.

For the drinking test, food was removed from the home cage, and a calibrated drinking tube was clipped in position at the front of the cage. At hourly intervals over a 5 hr period, water intake was recorded (to the nearest 0.5 ml) by reading the water levels in the drinking tubes. The drinking test began at 11:30 a.m.

The results of the experiment were analysed using a 2-way ANOVA procedure for independent factors, and a t-test for independent groups.

RESULTS AND DISCUSSION

Injection of hypertonic saline stimulated drinking, much of the effect occurring within the first hour of access to water (Fig. 1). Rats challenged with the osmotic thirst stimulus (but not administered either chlordiazepoxide or naltrexone) drank 11.1±1.3 ml (mean±S.E. M.) in the first hour, and a

total of 14.6±1.7 ml by the end of the 5 hr period. These intake values were significantly in excess of the corresponding values of 4.9±0.2 ml and 5.5±0.5 ml for the control animals which received only isotonic saline injections (Fig. 1). Chlordiazepoxide (10 mg/kg) significantly enhanced the drinking elicited by the osmotic challenge, for each hourly interval of the test period. Chlordiazepoxide-treated rats drank a total of 27.8±3.6 ml by the end of the test (Fig. 1).

Figure 2 depicts the effects of naltrexone (0.3–3 mg/kg) on the drinking elicited by hypertonic saline in rats treated with either chlordiazepoxide or its vehicle, for the 5 hr test period. Analysis of variance revealed that naltrexone treatment produced a highly significant reduction in water consumption in rats challenged with the osmotic thirst stimulus, $F(3,48)=10.1$, $p<0.0001$, and confirmed that chlordiazepoxide significantly enhanced the drinking in animals challenged with the thirst stimulus, $F(1,48)=16.9$, $p<0.0001$. There was also a significant naltrexone × chlordiazepoxide interaction, $F(3,48)=2.9$, $p<0.05$. This reflected the greater effect of naltrexone in those rats treated with chlordiazepoxide, compared with its effect in animals treated with chlordiazepoxide vehicle (Fig. 2). In fact, naltrexone at 3 mg/kg completely abolished the chlordiazepoxide-enhancement of drinking, whilst only reducing the drinking due to the hypertonic saline treatment itself by a mean of 5.5 ml, or a 37.8% reduction. Hence naltrexone dose-dependently suppressed the chlordiazepoxide effect on drinking in rats challenged with hypertonic saline.

Previous work has shown that naloxone and naltrexone attenuate the drinking elicited by administration of hypertonic saline in rats and mice [5, 6, 9, 16, 31, 33], and the present results with naltrexone are therefore confirmatory. The present data extend other published work which has shown that chlordiazepoxide enhances water consumption in water-deprived rats [10, 11, 12, 14, 28, 29, 30, 34, 35], by demonstrating that it can also facilitate the drinking which is elicited by cellular dehydration alone. Most importantly, the present experiment showed that naltrexone treatment could completely suppress the chlordiazepoxide-enhancement of drinking in osmotically-challenged rats. This interesting result extends the earlier observation that opiate receptor antagonists abolish the hyperdipsic action of chlordiazepoxide in water-deprived rats [12].

EXPERIMENT 2

METHOD

The subjects were a second batch of 63 male hooded rats from our laboratory. They were maintained under the conditions used in the first experiment, and were within a similar weight range. Again they were allocated at random to nine equal groups, and each rat received three injections prior to a 5 hr drinking test. Animals in eight of the groups were lightly etherised and injected subcutaneously with 5 ml 20% (w/w) polyethylene glycol (PEG, mol. wt. 20,000), obtained from Sigma, London. This injection was administered 2 hr before the start of the drinking test. The second and third injections were arranged as in the first experiment, when chlordiazepoxide or its vehicle was administered 40 min before the test, and naltrexone (0.1–1 mg/kg) 20 min before the test. Pilot work had indicated that a slightly lower dose range was applicable in PEG-treated rats. Rats in the ninth group were also lightly etherised 2 hr before the test, but were given 3 injections of isotonic saline at the appropriate times before the test. Their data provided the baseline drinking scores, against which to compare the effects of the 20% PEG treatment.

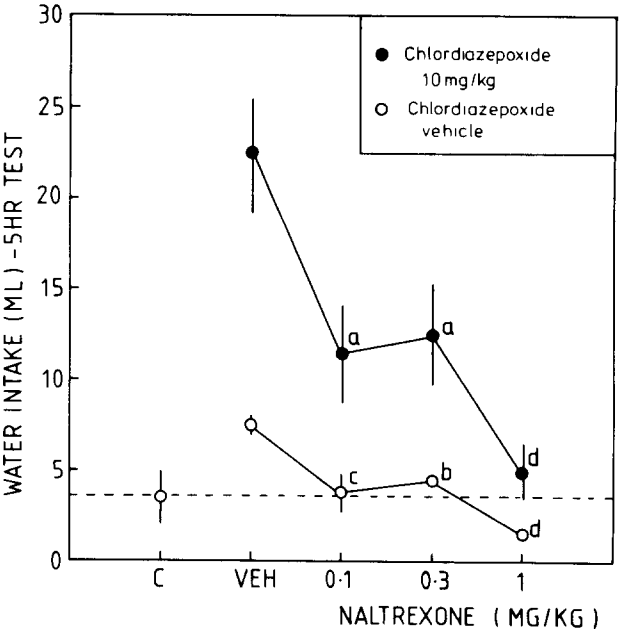

FIG. 3. Antagonism of chlordiazepoxide-enhancement of hypovolemic thirst elicited by 20% PEG after naltrexone (0.1–1 mg/kg). Other details are as described for Fig. 2.

In the drinking test, food was removed from the home cage, and a calibrated drinking tube was clipped into position. Water intake (ml) was measured at hourly intervals over a 5 hr period, and the test began at 11:30 a. m. The results of the experiment were analysed as were those in the first experiment.

RESULTS AND DISCUSSION

Figure 3 shows the effects of naltrexone (0.1–1 mg/kg) on the total water intake (ml) elicited by 20% PEG in rats treated with either chlordiazepoxide or its vehicle, over the first 5 hr period. Depletion of extracellular fluid volume by PEG produced a significant drinking response compared to that of control animals injected with an equivalent volume of isotonic saline, $t(12)=2.50$, $p<0.025$. Chlordiazepoxide (10 mg/kg) significantly enhanced the drinking elicited by PEG injection, $t(12)=4.36$, $p<0.005$, raising the 5 hr water intake from 7.5±0.6 ml to 22.4±3.1 ml.

An analysis of variance of the complete data for PEG-treated animals confirmed that naltrexone significantly attenuated water consumption, $F(3,48)=6.2$, $p<0.005$. Chlordiazepoxide produced a highly significant increase in drinking, $F(1,48)=19.0$, $p<0.0001$. The naltrexone × chlordiazepoxide interaction was not significant ($F<1.0$). Naltrexone produced a substantial reduction in drinking in animals treated with chlordiazepoxide or the chlordiazepoxide vehicle (Fig. 3). At 1 mg/kg, naltrexone reduced the level of water consumption in chlordiazepoxide-treated rats to a value that was not statistically different from the baseline level of drinking, $t(12)=0.61$, N.S.) At a dose as small as 0.1 mg/kg, naltrexone reduced water intake in rats

injected with 20% PEG and chlordiazepoxide vehicle to a level which was equivalent to the baseline drinking ($t=0.09$).

Investigators have previously shown that drinking in response to the hypovolemic thirst stimulus produced by PEG injection is attenuated by naloxone [31,33]. Rowland [33] reported a partial attenuation of drinking elicited by 20% or 30% PEG following the administration of naloxone (0.1–5 mg/kg). In the present experiment, naltrexone (0.1–1 mg/kg) reduced drinking to baseline values and below, thus suppressing completely the response to PEG. This quantitative distinction is in agreement with other data which indicate a somewhat greater potency of naltrexone compared with naloxone in drinking experiments [7]. The present study demonstrated a clear enhancement of drinking in PEG-treated rats by chlordiazepoxide (10 mg/kg). This chlordiazepoxide effect on drinking was completely abolished by naltrexone at 1 mg/kg, and attenuated at lower dose values.

GENERAL DISCUSSION

Some of the most characteristic behavioral effects of the benzodiazepines are attenuated by the action of opiate antagonists. Thus the anticonflict effects of diazepam and chlordiazepoxide [2, 4, 17, 36], the enhanced lateral hypothalamic self-stimulation which follows chlordiazepoxide administration [27], and diazepam-induced hyperphagia [3, 4, 36, 37] are reversed by naloxone. These findings have been extended to show that chlordiazepoxide-induced hyperdipsia in water-deprived rats was completely blocked by small doses of either naloxone or naltrexone [12]. The specificity of the interaction was confirmed by the result that an equivalent phenobarbital-induced hyperdipsia was not diminished to any significant degree by either naloxone or naltrexone [13]. The two experiments of the present report showed first that chlordiazepoxide stimulated water consumption in rats challenged with either a cellular thirst stimulus (hypertonic saline) or with an extracellular thirst stimulus (20% PEG). Second, they showed that naltrexone at 3 mg/kg completely attenuated the chlordiazepoxide effect in osmotically-challenged rats (Fig. 2), and at 1 mg/kg removed the benzodiazepine effect in hypovolemically-challenged rats (Fig. 3). Taken together therefore, the data of the present study and of previous experiments clearly show that opiate antagonists attenuate at least some of the typical behavioral effects of benzodiazepines. They raise the important possibility that the release of endogenous opioid peptides may necessarily be involved in these effects.

At a biochemical level, there is evidence to support this view. Acute diazepam treatment produces a naloxone-reversible decrease in striatal enkephalin content, probably due to an increased release of enkephalin [18,39]. There is strong evidence that benzodiazepines enhance γ-amino butyric acid (GABA) neurotransmission [15, 25, 26], and it is significant therefore that the effect of diazepam on striatal enkephalin levels appears to be GABA-mediated [19]. These data suggest a model of benzodiazepine action which depends on GABAergic mechanisms, and then on naloxone-reversible enkephalin release. This model can be applied in an attempt to understand the relationship between benzodiazepine mechanisms and the response to thirst in the rat. Benzodiazepines promote excess drinking, not only in water-deprived rats but also in rats challenged with an osmotic thirst stimulus (Experiment 1) or a hypovolemic thirst stimulus (Experiment 2). Benzodiazepines may therefore act upon the drinking response to thirst, rather than on one or other thirst signal. Chlordiazepoxide blocks the hypodipsic effects of GABA antagonists [10,11], and in turn, the hyperdipsic effects of chlordiazepoxide are suppressed by naloxone and naltrexone ([12]; this study). Future research should be directed to determine if striatal enkephalin release is directly involved in the facilitatory action of benzodiazepines on drinking responses.

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